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DESCRIPTION

INTRAVAGINAL WASHING AGENT

5 Technical Field

The present invention relates to a washing agent to wash the vagina of a patient suffering from a vaginal infection such as bacterial vaginosis, chlamydial cervicitis, trichomonal vaginitis, or candidal vaginitis, the washing
10 agent also being effective as a curative medicine, and to medical care and pharmaceutical technology.

Background Art

A large number of fungi of the genus lactic acid
15 bacillus such as Doderlein's bacillus reside in the vagina of healthy adult women who menstruate. The presence of these lactic acid bacilli maintains the healthy balance of bacteria in the vagina and prevents the reproductive organ from being infected by pathogenic microbes.

20

The lactic acid bacillus is reproduced using glycogen secreted from the vaginal mucosa as an energy source and competes with external pathogens to protect the vaginal cavity from attack by other bacteria. However, because of
25 the recent development of a large number of antibiotics,

pharmaceutical preparations of such antibiotics have often been used, causing the lactic acid bacilli in the vagina to be eliminated.

5 As a result, a large number of infected anaerobic bacteria such as Bacteroides, Peptostreptococcus, Peptococcus, and Mobiluncus G. vaginalis are reproduced in the vagina and interaction between these bacteria causes the development of bacterial vaginitis. Also, the infection of
10 Trichomonas vaginalis or Candida (all of the above Candida albicans) causes the development of vaginitis.

Sulfonamides, antibiotics, and antibacterial agents are chiefly used to cure the above diseases. However, there is
15 a concern that the administration of such antibiotics causes side effects such as rash, pruritus, local reddening, inflammation, and erosion. The administration of such antibiotics also leads to the death of the lactic acid bacilli indwelling in the vagina. Furthermore, since some
20 pathogenic bacteria (for example, Staphylococcus) acquire resistance to the antibiotics, it has been difficult to cure such diseases by administering antibiotics.

Japanese Unexamined Patent Application Publication No.
25 11-322621 discloses the use of lactic acid bacillus such as

Lactobacillus casei, Lactobacillus gasseri, Lactobacillus
fermintum, Lactobacillus casei subs. Pseudopiantarum, and
Lactobacillus crispatus to cure vaginal infections. This
seems to be based on an idea that the lactic acid bacillus
5 is used to replenish the dead bacilli.

Because of the overuse of antibiotics, the decrease in
the immune system's function, and the increase in sexual
behavior, the number of women suffering from a disease such
10 as bacterial vaginosis, chlamydial cervicitis, trichomonal
vaginitis, or candidal vaginitis has been increasing. Many
women are reluctant to visit a gynecologist for such a
disease and the symptoms are often aggravated.

15 In order to improve such a situation, the present
inventor has considered the known art, developed a washing
agent, and performed studies so that the patient can cure
the disease by herself, in other words, the patient can wash
the vagina by herself. The patient can recover the self-
20 cleaning effect in the vagina using this washing agent in
the home. The washing agent prevents bacteria, fungi, and
protozoa, etc. from invading into the vagina and sterilizes
the invading microorganisms. The washing agent can be
easily handled, has no toxicity, and exhibits excellent
25 effects.

As a result, the present inventor has found that substances generally produced and secreted by lactic acid bacteria (hereinafter referred to as an extract of
5 fermentation solution of lactic acid bacteria), which have an effect of improving the condition of intestinal microorganisms, and which are commercially available, solve the above problems. The present inventor has also found that the above substances can be used as an excellent
10 washing agent of the vagina for a woman suffering from bacterial vaginosis, chlamydial cervicitis, trichomonal vaginitis, or candidal vaginitis, and the above substances are effective as a curative medicine for such a vaginal infection. Thus, the present inventor has accomplished the
15 present invention.

Disclosure of Invention

The invention described in claim 1 of the present
20 invention provides

an intravaginal washing agent composed of fermented soybean milk produced by fermenting soybean milk with a co-culture of a plurality of lactic acid bacteria.

25 The invention described in claim 2 of the present

invention provides

an intravaginal washing agent composed of an extract
produced by extracting fermented soybean milk with an
alcohol, the fermented soybean milk being produced by
5 fermenting soybean milk with a co-culture of a plurality of
lactic acid bacteria.

The invention described in claim 3 of the present
invention provides

10 the intravaginal washing agent composed of an extract
produced by the following process. The process includes the
steps of sterilizing the fermented soybean milk produced by
fermenting soybean milk with a co-culture of a plurality of
lactic acid bacteria, adding an alcohol to the fermented
15 soybean milk, and performing the extraction with the alcohol
for at least six months.

The invention described in claim 4 of the present
invention provides

20 the intravaginal washing agent composed of an extract
produced by extracting fermented soybean milk with an
alcohol, the fermented soybean milk being produced by
fermenting soybean milk with a co-culture of a plurality of
lactic acid bacteria,

25 wherein the extract includes a total content of at

least 20% of an ingredient of which the retention time measured by liquid chromatography under the following conditions is at least 10 minutes.

Measurement conditions for liquid chromatography

5 Column: Asahipak GS=220H
 Mobile phase: 100 mM sodium phosphate buffer
 Flow rate: 1.0 /min.
 Column temperature: 40° C

10 The invention described in claim 5 of the present invention provides

 the intravaginal washing agent composed of an extract produced by extracting fermented soybean milk with an alcohol, the fermented soybean milk being produced by
15 fermenting soybean milk with a co-culture of a plurality of lactic acid bacteria,

 wherein the extract includes a total content of at least 25% of an ingredient of which the retention time measured by liquid chromatography under the following
20 conditions is at least 7 minutes.

Measurement conditions for liquid chromatography

 Column: Asahipak GS=220H
 Mobile phase: 100 mM sodium phosphate buffer
 Flow rate: 1.0 /min.
25 Column temperature: 40° C

The invention described in claim 6 of the present invention provides

the intravaginal washing agent composed of an extract
5 produced by extracting fermented soybean milk with an alcohol, the fermented soybean milk being produced by fermenting soybean milk with a co-culture of a plurality of lactic acid bacteria,

wherein the extract includes a total content of at
10 least 25% of an ingredient of which the retention time measured by liquid chromatography under the following conditions is at least 7 minutes, and a total content of at least 20% of an ingredient of which the retention time measured by liquid chromatography under the following
15 conditions is at least 10 minutes.

Measurement conditions for liquid chromatography

Column: Asahipak GS=220H

Mobile phase: 100 mM sodium phosphate buffer

Flow rate: 1.0 /min.

20 Column temperature: 40°C

Brief Description of the Drawings

Fig. 1 is a chart from a liquid chromatograph of an extract of fermentation solution of lactic acid bacteria
25 according to the present invention.

Best Mode for Carrying Out the Invention

An intravaginal washing agent according to the present
5 invention includes fermented soybean milk as the active
ingredient. The fermented soybean milk is produced by
fermenting soybean milk with a co-culture of a plurality of
lactic acid bacteria, preferably, for about 100 to about 300
hours. This fermented soybean milk itself can be used as
10 the intravaginal washing agent in the present invention.

More specifically, examples of the active ingredient of
the intravaginal washing agent include a substance
containing an extract of fermented soybean milk and other
15 various impurities, and a solid or a liquid prepared by
solid-liquid separation and containing the extract of
fermented soybean milk. In order to utilize the extract of
fermented soybean milk effectively, the extract used as the
effective ingredient is preferably extracted with an organic
20 solvent. More preferably, the extract is produced by
extracting fermented soybean milk with an alcohol, the
fermented soybean milk being produced with a co-culture of a
plurality of lactic acid bacteria.

25 It is known that substances generally produced and

secreted by lactic acid bacteria, which are contained in fermented soybean milk produced by fermentation with a co-culture (hereinafter referred to as fermented soybean milk extract), have a superior advantage for human health. In addition, many products containing such substances are commercially available.

The fermented soybean milk extract in the present invention is obtained from fermented soybean milk produced by fermenting soybean milk with a co-culture of a plurality of lactic acid bacteria, and in particular, is obtained by extracting the fermented soybean milk with an alcohol. The conditions for the extraction with an alcohol are as follows:

- (1) The alcohol is preferably ethanol because the fermented soybean milk extract is used as a food. An approximately equivalent amount of ethanol is added to the fermented soybean milk, and the fermented soybean milk is extracted.
- (2) Sterilized fermented soybean milk is used for the extraction. Examples of the sterilization method include heat sterilization and sterilization by disrupting the bacterial cells.

Although the extraction may be performed with heating, the extraction is preferably performed at normal temperature

to prevent thermal denaturation. In addition, the extraction is preferably performed in a cool, dark place to prevent the long-term influence of light.

5 It is expected that the extraction at normal temperature proceeds at a low speed. In order to produce a superior extract from the fermented soybean milk, the extraction is performed for a long time, generally for at least 6 months, and preferably at least 1 year. Taking such
10 a long time provides a superior fermented soybean milk extract.

 Fermented soybean milk produced by fermentation with a co-culture of a plurality of lactic acid bacteria is
15 sterilized, and an alcohol is then added to the fermented soybean milk. The extraction is performed for at least 6 months. As will be clarified in the following Examples, this process provides the following fermented soybean milk extract and also increases the kind of the ingredient. The
20 fermented soybean milk extract includes a total content of at least 20%, preferably at least 25%, of an ingredient of which the retention time measured by liquid chromatography under a specific condition is at least 10 minutes.

25 The ingredient is defined in more detail as follows:

The fermented soybean milk extract includes a total content of at least 25%, preferably at least 30%, of an ingredient of which the retention time measured by liquid chromatography is at least 7 minutes; or the fermented

5 soybean milk extract includes a total content of at least 25%, preferably at least 30%, of an ingredient of which the retention time measured by liquid chromatography is at least 7 minutes and a total content of at least 20%, preferably at least 25%, of an ingredient of which the retention time
10 measured by liquid chromatography is at least 10 minutes.

As the extraction is performed for a long time, the amount of each ingredient in the fermented soybean milk extract gradually increases. However, since the long-term extraction decreases the extraction effect and decreases the
15 efficiency of the extraction operation, a fermented soybean milk extract generally containing each ingredient up to 40% and up to 35%, preferably containing up to 35% and up to 30% is used.

20 In order to clean the vaginal walls, the fermented soybean milk or the extract thereof that is the intravaginal washing agent of the present invention can be used as follows without further treatment or it can be diluted. The fermented soybean milk or the extract thereof may be applied
25 on the pudendum. For example, gauze soaked with the

fermented soybean milk or the extract thereof may be applied on the affected area. Furthermore, the fermented soybean milk or the extract thereof may be introduced and dispersed in the vagina with a dropping pipette, and a cotton ball or
5 a tampon may then be inserted for some time.

The fermented soybean milk or the extract thereof in the present invention does not impose a strict limitation on the quantity applied, and can be freely used, because it is
10 widely ingested as a health maintenance food and does not cause side effects. Therefore, the intravaginal washing agent of the present invention may be used everyday, every other day, or every few days. As a result, the use of the intravaginal washing agent cleans the inside of the vagina
15 to alleviate or terminate the symptoms of, for example, bacterial vaginosis, chlamydial cervicitis, trichomonal vaginitis, or candidal vaginitis.

<EXAMPLES>

20 The intravaginal washing agent of the present invention will now be described in more detail with reference to the Examples.

EXAMPLE 1

<Preparation extract of fermentation solution>

25 As shown in the following Table 1, lactic acid bacteria

and yeast that form combinations of four kinds and four groups were prepared. In the table, symbols A, B, 1, 2, 3, and 4 represent that the strain is different.

5 Table 1

	(1)	(2)	(3)	(4)
I	B. bulgaricus A	B. acidophilus 1.	Micrococcus lactisacidi 1.	Yeast 1.
II	B. bulgaricus B	B. acidophilus 2.	Micrococcus lactisacidi 2.	Yeast 2.
III	Kornchenbacillus A	B. acidophilus 3.	Micrococcus lactisacidi 3.	Yeast 3.
IV	Kornchenbacillus B	B. acidophilus 4.	Micrococcus lactisacidi 4.	Yeast 4.

The lactic acid bacteria in each group were separately cultured for 48 hours using soybean milk as the culture medium, while the temperature was gradually increased from 20° C to 40° C.

After the culture was performed for 48 hours, the culture solutions were transferred to one container. The mixture was further cultured for 96 hours, while the temperature was gradually increased from 20° C to 40° C.

The fermented soybean milk prepared by completing the culture was sterilized by heating. Subsequently, an equivalent amount of alcohol was added to the solution. The mixture was kept in the cool and dark place for one year to perform the extraction.

The extracted fermented soybean milk was filtered and subjected to solid-liquid separation to prepare a clear light yellow fermented soybean milk extract I.

The resultant extract of fermentation solution was analyzed by liquid chromatography under the following conditions. Table 2 and Fig. 1 show the results.

Measurement conditions for liquid chromatography

Column: Asahipak GS=220H

Mobile phase: 100 mM sodium phosphate buffer

Flow rate: 1.0 /min.

Column temperature: 40°C

Detector: Ultraviolet spectrophotometer

Detection wavelength: 210 nm

Table 2

ingredient	retention time	area	height	concentration
1	5.194	8974013	71553	37.8998
2	6.815	7031508	73112	29.6960
3	9.382	1118511	19495	4.7238
4	10.521	559947	14058	2.3648
5	12.199	2181670	17105	9.2138
6	14.993	908867	7338	3.8384
7	18.435	445615	3782	1.8820
8	21.474	362632	4422	1.5315
9	23.559	1158857	10852	4.8942
10	26.911	237078	2011	1.0012
11	29.886	358612	2975	1.5145
12	32.622	340970	2516	1.4400
total		23678272	229218	100.0000

<Confirmatory test of cleaning effect-1>

After written informed consent was obtained from seven
5 female outpatients (19 years old to 29 years old) who
visited a gynecologist complaining of an increase in vaginal
discharge and an unpleasant odor therefrom, 1 cc of the
above extract of fermentation solution was evenly dispersed
on the vaginal walls with a dropping pipette under the
10 direct observation of the inside of the vagina. Subsequently,
a cotton ball sterilized by high pressure was inserted in
the vagina. Four hours later, the cotton ball was removed
by the patients.

When the intravaginal cleaning was performed, vaginal
15 discharge in the vagina was collected to check for Chlamydia,
Trichomonas, and Candida. Patients who were negative for
Chlamydia, Trichomonas, and Candida, and had vaginal
discharge that was yellow and had an amine-like odor were
clinically diagnosed as having bacterial vaginosis. Among
20 the seven patients, five women were diagnosed with bacterial
vaginosis, one woman was diagnosed with chlamydial
cervicitis, and one woman was diagnosed with candidal
vaginitis.

25 The cleaning effects in the patients were as follows.

The duration of the test was March to April in 2002.

(1) Patient (A.M.) 20 years old, Bacterial vaginosis

March 28, First cleaning, A large amount of yellow vaginal discharge, Strong odor

5 March 30, Second cleaning, Both the vaginal discharge and the odor were somewhat decreased. (Two days after the first cleaning)

10 April 6, Third cleaning, Both the vaginal discharge and the odor were improved by 100%. (Eight days after the first cleaning)

 April 9, Fourth cleaning, The condition in which both the vaginal discharge and the odor were improved by 100% was maintained.

15 (2) Patient (F.Y.) 29 years old, Bacterial vaginosis

 April 9, First cleaning, A large amount of yellow vaginal discharge, Strong odor

 April 11, Second cleaning, Remained the same, (Two days after the first cleaning)

20 April 13, Third cleaning, Both the vaginal discharge and the odor were improved by 50%. (Four days after the first cleaning)

25 April 15, Fourth cleaning, The condition in which both the vaginal discharge and the odor were improved by 50% was maintained.

(3) Patient (Y.E.) 19 years old, Bacterial vaginosis

April 9, First cleaning, A large amount of yellow vaginal discharge, Strong odor

5 April 11, Second cleaning, The vaginal discharge and the odor remained the same, (Two days after the first cleaning)

April 15, Third cleaning, The vaginal discharge was decreased by 30%, and the odor was improved by 40%.

10

(4) Patient (Y.C.) 24 years old, Bacterial vaginosis

March 8, First cleaning, A large amount of yellow vaginal discharge, Strong odor

March 12, Second cleaning, Both the vaginal discharge and the odor were somewhat decreased. (Four days after the first cleaning)

March 15, Third cleaning, The vaginal discharge was improved by 50%, and the odor was improved by 100%.

March 22, Fourth cleaning, The condition in which the vaginal discharge was improved by 50% and the odor was improved by 100% was maintained.

(5) Patient (K.H.) 29 years old, Bacterial vaginosis

March 5, First cleaning, A large amount of yellow vaginal discharge, Strong odor

25

March 11, Second cleaning, Both the vaginal discharge and the odor were somewhat decreased. (Six days after the first cleaning)

March 14, Third cleaning, The vaginal discharge and the
5 odor were further improved. (Nine days after the first cleaning)

March 22, Fourth cleaning, Both the vaginal discharge and the odor were improved by 100%.

10 (6) Patient (M.S.) 21 years old, Chlamydial cervicitis

April 6, First cleaning, A large amount of yellow vaginal discharge, Strong odor

April 8, Second cleaning, Both the vaginal discharge and the odor were somewhat decreased. (Two days after the
15 first cleaning)

April 9, Third cleaning, The vaginal discharge was improved by 40%, and the odor was improved by 100%.

April 12, Fourth cleaning, The condition in which the vaginal discharge was improved by 40% and the odor was
20 improved by 100% was maintained.

(7) Patient (S.M.) 27 years old, Candidal vaginitis

March 8, First cleaning, A large amount of white vaginal discharge

25 March 11, Second cleaning, The vaginal discharge was

somewhat decreased. (Three days after the first cleaning)

March 14, Third cleaning, The vaginal discharge was decreased by 80%. (Six days after the first cleaning)

April 15, Fourth cleaning, The condition in which both
5 the vaginal discharge and the odor were improved by 50% was maintained.

<Confirmatory test of cleaning effect-2>

The following symptoms were tested by outpatients who
10 mainly complained of vaginal discharge with an unpleasant odor.

1. Gray vaginal discharge
2. pH in the vagina > 4.5 (5.0)
3. Detection of an amine-like odor
- 15 4. Detection of a clue cell (at least 20% of the epithelial cells)

Among 30 patients who were positive for the three items out of the above symptoms, 23 patients who could visit the hospital for treatment for one week were selected. After
20 written informed consent was obtained from the patients, the above-mentioned extract of fermentation solution was administered in the vagina. On the other hand, Chlomy (registered trademark) vaginal tablets (chloramphenicol vaginal tablets) were administered to seven patients who
25 could not visit the hospital.

The degree of the unpleasant odor and the vaginal discharge was evaluated by the test subjects as outpatients using a visual analog scale (VAS).

Table 3 shows the evaluation results of the patients to whom the extract of fermentation solution was administered. Table 4 shows the evaluation results of the patients to whom Chlomy vaginal tablets were administered. The evaluation showed the following results.

(1) In the patient group to whom the extract of fermentation solution was administered in the vagina, one week after the administration, the degree of the unpleasant odor and the vaginal discharge was significantly decreased ($P < 0.001$ and $P < 0.001$, respectively), compared with that before the administration.

(2) In the patient group to whom Chlomy vaginal tablets were administered, one week after the administration, the degree of the unpleasant odor and the vaginal discharge was significantly decreased ($P = 0.0016$ and $P = 0.0018$, respectively), compared with that before the administration.

(3) The change in the degree of the unpleasant odor and the vaginal discharge (decreasing ratio %) was represented by formula $[(\text{before administration} - \text{after administration}) / \text{before administration}]$. In the above decreasing ratio, there was no statistically significant difference between the group to whom the extract of fermentation solution was

administered in the vagina and the group to whom Chlomy vaginal tablets were administered.

(4) In conclusion, the administration of the fermentation solution extract in the vagina had the same curative effect as that of Chlomy vaginal tablets and caused no side effects.

Table 3

patient	age	first examination		one week later	
		vaginal discharge	unpleasant odor	discharge /decreasing rate(%)	odor /decreasing rate(%)
1	20	100	95	22/ 78	18/ 81
2	35	75	79	23/ 69	38/ 52
3	25	60	84	21/ 65	0/ 100
4	25	64	64	18/ 72	6/ 91
5	35	76	48	31/ 59	19/ 60
6	24	33	70	4/ 88	6/ 91
7	22	75	79	23/ 69	38/ 52
8	20	100	30	22/ 78	26/ 13
9	20	100	95	22/ 78	18/ 81
10	21	43	42	3/ 93	0/ 100
11	18	54	67	0/ 100	0/ 100
12	28	70	91	46/ 34	61/ 33
13	20	54	67	0/ 100	0/ 100
14	21	52	65	0/ 100	0/ 100
15	25	72	21	21/ 71	5/ 76
16	25	62	65	0/ 100	0/ 100
17	25	60	84	0/ 100	0/ 100
18	38	60	63	0/ 100	0/ 100
19	27	61	64	12/ 80	0/ 100
20	20	72	76	0/ 100	0/ 100
21	21	54	67	22/ 59	0/ 100
22	25	76	48	0/ 100	0/ 100
23	37	54	49	4/ 93	4/ 92

Table 4

patient	Age	first examination		one week later	
		vaginal discharge	Unpleasant odor	discharge /decreasing rate(%)	odor /decreasing rate(%)
1	23	73	65	12 / 84	10 / 84
2	28	91	70	21 / 77	42 / 40
3	22	79	75	14 / 82	11 / 85
4	18	47	55	17 / 64	34 / 38
5	37	100	71	0 / 100	23 / 68
6	33	64	75	15 / 77	45 / 40
7	21	58	50	43 / 26	34 / 32
8	21	91	92	1 / 99	0 / 100

Industrial Applicability

Ingredients in fermented soybean milk produced by

5 fermenting soybean milk with a co-culture of a plurality of lactic acid bacteria, particularly in the above fermented soybean milk extract have been identified to a certain degree. Although nucleic acids, amino acids, and vitamins are listed as the ingredients, some ingredients are still
10 unknown.

The following function of the extract of fermented soybean milk is known: The extract of fermented soybean milk improves the condition of intestinal microorganisms and
15 helps, for example, the synthesis of hormones, the synthesis of vitamins and enzymes, metabolism of cholesterol, blood sugar regulation, and blood pressure homeostasis. In

addition, the extract of fermented soybean milk is effective against hepatitis, chronic arthritis, atopic dermatitis, angina pectoris, and various cancers. However, only a few pharmacologic effects are specifically confirmed under the present situation. At present, the mechanism of action of the effects in the present invention is also not known.

However, as shown in the above clinical results, the fermented soybean milk or the extract thereof that is the intravaginal washing agent of the present invention is an effective medical agent (washing agent) against bacterial vaginosis, chlamydial cervicitis, and candidal vaginitis, and in addition, the effect works immediately. About three days after the administration, the symptoms were clearly improved and this condition was maintained. Even when the interval of the administration was one week or more, the effect could be maintained. Thus, the fermented soybean milk or the extract thereof is a superior medical agent.

These advantages are significantly excellent, compared with a known tablet of lactic acid bacteria having an effective ratio of about 60% and a known yogurt containing lactic acid bacteria having an effective ratio of about 50%, the yogurt causing incongruity or discomfort when used as a washing agent. When the curative effect was finally

assessed 30 days later, the fermented soybean milk or the extract thereof had excellent effects, compared with, for example, lactic acid produced by lactic acid bacteria. In this case, there was also a significant difference in the improvement in the surface of the skin.

In addition, the fermented soybean milk or the extract thereof that is the intravaginal washing agent in the present invention had the same curative effect as that of commercially available Chlomy vaginal tablets and had no side effects.

As clearly described above, the substances generally produced and secreted by lactic acid bacteria (hereinafter referred to as an extract of fermentation solution of lactic acid bacteria), which have an effect of improving the condition of intestinal microorganisms, and which are commercially available, can be used as an excellent washing agent of the vagina of a woman suffering from bacterial vaginosis, chlamydial cervicitis, trichomonal vaginitis, or candidal vaginitis. Furthermore, the above substances are effective as a curative medicine for such a vaginal infection. These substances can be widely used in the field of medical care, such as in medicines and pharmaceuticals.